and for "bound" biotin, *i.e.*, biotin which was liberated after 18 hours digestion of the feces at 100° C. in 20 per cent. sulfuric acid. Although there were some irregularities in the assays, it was found that both groups of chicks were excreting from 10 to 20 per cent. of their biotin intake as free biotin and approximately 15 to 25 per cent. additional as "bound" biotin. On an actual weight basis, the injured chicks were, of course, excreting more than the controls, since their intake was greater.

By the eighth week, the usual syndrome had become very pronounced in the injured group, so the tissues of two chicks from each group were then assayed for their biotin content; two weeks later tissues from an additional chick of each group were tested. These tissues were allowed to autolyze under toluene for three days at 37° C., after which they were thoroughly extracted with hot water. The tissues from the injured chicks were found to be consistently lower in their biotin content than were those from the control chicks, as can be seen from the tabulation of the assay values (Table 1).

These preliminary results indicate that the biotin which is present in the diet of the injured chicks (and which is more than sufficient in the absence of egg white) is not available to the tissues. Presumably it is destroyed by interaction with the egg white, and therefore an excess of biotin must be present in a diet containing egg white in order for the tissues to receive the necessary amount. It is probable that the injury caused by egg white is not due to any direct toxin, but rather is produced indirectly by the action of the egg white in making the biotin of the diet unavailable. If such is the case, it should be possible to produce similar syndrome by a diet which is actually deficient in biotin, but which contains no egg white.

TABLE 1

BIOTIN CONTENT OF TISSUES IN γ PER GRAM

Diet: Age:	Injury 8 wks.	Injury 8 wks.	Injury 10 wks.	Control 8 wks.	Control 8 wks.	Control 10 wks.
Blood	0.0018	0.0021		0.0051	0.0067	0.0042
Liver	0.95	0.58	0.60	2.5	2.8	2.6
Kidney	0.45	1.3	1.0	1.9	1.8	2.5
Heart	0.018	0.041	0.036	0.11	0.033	0.11
Brain	0.025	0.029	0.044	0.067	0.018	0.065
Leg muscle.	0.008	0.016	0.015	0.027	0.018	0.033

We wish to thank Dr. T. H. Jukes for his kind cooperation in furnishing advice and certain materials for these experiments.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MOLECULAR WEIGHT BY ISOTHERMIC DISTILLATION

OF all the solution methods for the determination of molecular weight of organic substances, the ingenious method of G. Barger[1] offers the widest applicability. This method is based upon the fact that a solution of higher molarity takes up solvent from a solution of lower molarity and *vice versa* until an equilibrium is reached. In a closed system, this produces changes in the volumes of a given standard solution of known molarity and the solution of the unknown substance but of known concentration. In practice, these changes in volume are determined by measuring at certain time intervals the diameter of several droplets contained in a sealed capillary and containing alternately the standard solution (*st*) and the solution of the unknown (*s*) in the same solvent which need not be pure (ethyl alcohol, pyridine, etc.). By appropriately choosing the molarity of the standard until the least changes are noted, the molecular weight of the unknown, the concentration of which, however, is known, may be readily calculated $\left(\frac{\%\ s}{M\ st} \times 10\right)$.

[1] G. Barger, *Jour. Chem. Soc.*, 85: 286, 1904; *Ber.*, 37: 1754, 1904.

The greatest drawback of this method as well as its various modifications[2] is that the droplets, either in the filling operation or subsequently on standing, frequently undergo mixing, thus invalidating the determination.

It has now been found that this objection, namely—the mixing—is readily overcome by having the two solutions, the standard and the unknown, in two separate capillaries of about 7–8 cm in length and 1–1.5 mm in diameter (Fig. 1, A and B). The capillaries

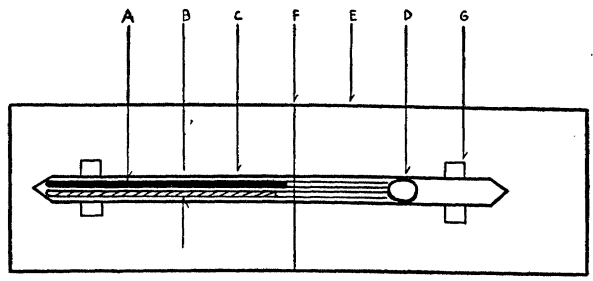

FIG. 1. Apparatus for isothermic distillation.

are filled by drawing up by suction the respective solutions, while they are open at both ends. Then the

[2] J. B. Niederl and V. Niederl, "Micromethods of Quantitative Organic Elementary Analysis," pp. 184–186. New York, N. Y.: J. Wiley and Sons. 1938.

capillary orifice furthest away from the solution is sealed in a micro burner. After cooling, the solution is centrifuged towards this sealed end. The two capillaries thus filled are then placed in a suitable glass tubing about 10 cm in length and 5 mm in diameter (C). They are put in place by means of a wad of glass wool or cotton (D). The tube is then evacuated to about 15 mm pressure and is then sealed by means of an ordinary Bunsen burner. The tube may once more be subjected to centrifuging in order to have a clean-cut meniscus of each of the solutions contained in the capillaries. By means of adhesive tape (G) the tube is then attached to a rectangular glass plate, which is about 12 cm long and about 4 cm wide (E) and which possesses a hairline or a scratch across its width (F). The plate thus mounted is then placed into a water bath which is kept at room temperature to within $\pm 3°$ C. After four days, the distance between the two menisci of the two solutions and the scratch, or hairline on the plate, is measured under a low-powered microscope possessing a micrometer scale in the eyepiece. These measurements are repeated subsequently once a day for a week. Thus it is easily ascertained which of the two solutions lost less solvent, this being the solution of higher molarity.

The molarities of the standard (azobenzene) employed are 0.05, 0.1 and 0.15 and the most suitable concentrations of the unknown sample are between 1 and 3 per cent. Differentiation between ± 0.01 molarities is possible.

The method described precludes any possibility of mixing of the two solutions and also permits the recovery and re-use of the unknown, while the results,[3] which will be published in detail in an appropriate analytical journal, compare favorably with the original Barger method or any of the known modifications thereof. The weighing out of the sample may be done on a macro, semi-micro or micro scale.

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A SIMPLE STAIN FOR TISSUE CULTURES

ANY one confronted with the necessity of staining hanging-drop tissue cultures grown in plasma has been impressed with the difficulty of securing a cytoplasmic stain which would not also tint the plasma of the clot to such intensity as to obscure largely the delicate cytoplasmic contours of the cells.

Many stains have been tried in this laboratory for coloring tissue cultures *in situ* in the plasma clot. The following simple method has given consistently good results on countless cultures over a period of nearly a year. It was developed to stain particularly cultures of brain tissue in which it reveals with a surprising clarity the finest processes of the cells as well as the nuclei and certain cytoplasmic inclusions.

[3] A. M. Levy, M.Sc. Thesis, New York University, Graduate School, April, 1940.

METHOD

(1) Remove all paraffin and vaseline from the coverslip with cotton pledgets soaked in chloroform.

(2) Fix in 10 per cent. neutral formalin or absolute alcohol for 24 hours.

(3) Place in 1 per cent. aqueous solution of Toluidine blue for 1 hour.

(4) Wash in two changes of distilled water.

(5) Dehydrate in 85 per cent. alcohol 2–3 minutes.

(6) Place in 95 per cent. alcohol 2–3 minutes.

(7) Transfer to absolute alcohol until the clot contains little stain. This step may be controlled by watching the decoloration under a microscope. The differentiation takes 5–10 minutes.

(8) Clear in xylol.

(9) Mount in balsam or Nevillite.

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BOOKS RECEIVED

BARKER, LEWELLYS F. *Psychotherapy.* Pp. ix + 218. Appleton-Century. $2.00.

Carnegie Institution of Washington. *Miscellaneous Papers, XIV–XXI; Botany of the Maya Area.* Pp. 474. Illustrated. The Institution.

CARROLL, PAUL L. and WILFRED F. HORNER. *An Atlas of the Frog.* Looseleaf. Illustrated. Mosby. $1.25.

DEAM, CHARLES C. *Flora of Indiana.* Pp. 1236. Illustrated. Department of Conservation, Division of Forestry, Indiana. $3.50.

GALDSTON, IAGO. *Progress in Medicine.* Pp. 347 + xiv. Knopf. $3.00.

GERARD, R. W. *Unresting Cells.* Pp. xv + 439. 173 figures. Harper. $3.75.

HUME, EDWARD H. *The Chinese Way in Medicine.* Pp. 189. Illustrated. Johns Hopkins Press. $2.25.

LEACH, JULIAN G. *Insect Transmission of Plant Diseases.* Pp. xviii + 615. 238 figures. McGraw-Hill. $6.00.

PRODINGER, WILHELM. *Organic Reagents Used in Quantitative Inorganic Analysis.* Translated from the second German edition by STEWART HOLMES. Pp. xiv + 203. Nordemann. $5.00.

SCHILLETTER, JULIAN C. and HARRY W. RICHEY. *Textbook of General Horticulture.* Pp. ix + 367. 136 figures. McGraw-Hill. $3.00.

SWIGERT, ARTHUR M. *The Story of Superfinish.* Pp. 672. 720 figures. Lynn, Detroit.

The Changing Front of Health. Proceedings of the Eighteenth Annual Conference of the Milbank Memorial Fund, April, 1940. Pp. 104. The Fund, New York.

University Mathematical Texts: AITKEN, A. C. *Determinants and Matrices.* Pp. 135. *Statistical Mathematics.* Pp. 153; GILLESPIE, R. P. *Integration.* Pp. 126; INCE, E. L. *Integration of Ordinary Differential Equations.* Pp. 148; RUTHERFORD, D. E. *Vector Methods.* Pp. 127; TURNBULL, H. W. *Theory of Equations.* Pp. 152. Interscience Publishers, New York. $1.50 each.

VIGOUREUX, P. *Quartz Oscillators and Their Applications.* Pp. vi + 131. 86 figures. His Majesty's Stationery Office, London. $1.35.

WERKMEISTER, WILLIAM H. *A Philosophy of Science.* Pp. xii + 551. 29 figures. Harper. $4.00.